MJCB
MALAYSIAN JOURNAL OF CLINICAL BIOCHEMISTRY

ORIGINAL ARTICLE

DECODING PORPHYRIA IN MALAYSIA: BIOCHEMICAL AND MOLECULAR CHARACTERISATION

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Received Date: *date (Please leave blank)
Revised Date: *date (Please leave blank)
Published Date: *date (Please leave blank)

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ABSTRACT

Porphyria is a group of rare inherited or acquired disorders arising from enzyme deficiencies in the heme biosynthesis pathway. Accurate diagnosis requires integration of clinical, biochemical, and genetic data to differentiate between subtypes. This study aimed to characterise porphyria subtypes among Malaysian patients using a multi-modal diagnostic approach involving urinary and plasma porphyrin analysis and molecular testing. Thirteen patients with clinical suspicion of porphyria underwent biochemical screening using high-performance liquid chromatography (HPLC) to analyse urinary and plasma porphyrins. Molecular investigations included whole-exome sequencing (WES), Sanger sequencing, and multiplex ligation-dependent probe amplification (MLPA) targeting key porphyria-related genes. All patients showed elevated urinary porphyrins, with levels correlating to clinical severity. Plasma porphyrin profiling supported diagnosis in acute intermittent porphyria (AIP) but was less informative for erythropoietic protoporphyria (EPP). Genetic analysis confirmed one AIP case with a pathogenic HMBS gene variant (c.673C>T), two cases suggestive of EPP with homozygous low-penetrance FECH gene variants (c.315-48T>C), and one case with a heterozygous pathogenic HFE gene variant (c.187C>G) linked to the risk of acquired porphyria cutanea tarda (PCT). Four cases had inconclusive genetic findings, while five showed no pathogenic variants, indicating the possibility of acquired porphyria or alternative diagnoses. This study highlights the value of combining biochemical and molecular tools in the diagnosis of porphyria. The presence of low-penetrance or absent variants in some patients underscores the need for thorough clinical evaluation and consideration of environmental triggers in suspected porphyria cases.

Keywords: *biochemical screening, FECH gene, genetic testing, HMBS gene, porphyria*

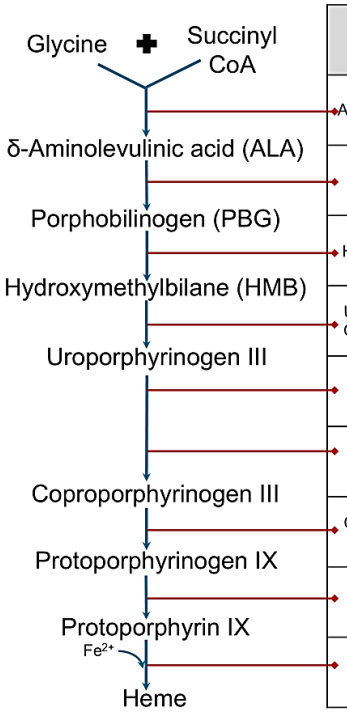
Abbreviations:

AD	: Autosomal Dominant
AIP	: Acute Intermittent Porphyria
ALAD	: Aminolevulinate Dehydratase
ALAS2	: 5-Aminolevulinate Synthase 2
AR	: Autosomal Recessive
CNV	: Copy Number Variation
CPOX	: Coproporphyrinogen Oxidase
D-ALA	: δ-aminolevulinic acid
DNA	: Deoxyribonucleic acid
EDTA	: Ethylenediaminetetraacetic acid
EPP	: Erythropoietic Protoporphyria
FECH	: Ferrochelatase
G6PD	: Glucose-6-Phosphate Dehydrogenase
HFE	: High Iron Fe (Hemochromatosis Gene)
HH	: Hereditary Hemochromatosis
HMBS	: Hydroxymethylbilane Synthase
HPLC	: High-Performance Liquid Chromatography
IMR	: Institute for Medical Research
MLPA	: Multiplex Ligation-dependent Probe Amplification
NGS	: Next Generation Sequencing
PBG	: Porphobilinogen
PBGD	: Porphobilinogen Deaminase
PCT	: Porphyria Cutanea Tarda
PPOX	: Protoporphyrinogen Oxidase
ROS	: Reactive Oxygen Species
SDC	: Specialised Diagnostic Centre
UROD	: Uroporphyrinogen Decarboxylase
UROS	: Uroporphyrinogen III Synthase
VP	: Variegate Protoporphyria
WES	: Whole Exome Sequencing
XLR	: X-linked Recessive

INTRODUCTION

Porphyria refers to a group of rare inherited or acquired disorders resulting from enzymatic defects within the heme biosynthetic pathway, and it continues to pose significant diagnostic challenges in clinical practice. These defects result in the accumulation of porphyrin precursors and intermediates, leading to a wide range of clinical manifestations, including neurovisceral symptoms, cutaneous photosensitivity, and hepatotoxicity (Neeleman et al., 2020). The clinical heterogeneity and overlapping features among porphyria subtypes often complicate the diagnosis process, leading to delayed or missed (Aarsand et al., 2024).

Globally, porphyrias are rare, with prevalence estimates ranging from 1–5 per 100,000 for acute hepatic porphyrias and 1 in 75,000–200,000 for erythropoietic protoporphyria (EPP) (Linenberger & Fertrin, 2020). However, in Malaysia, the true prevalence of porphyria is unknown due to the absence of national epidemiological data.



Enzymatic Involvement	Target Gene	Chromosomal Location	Inheritance	Type of Porphyria	Prevalence (case per million)*
ALA Synthase (ALAS)	*ALAS2 Gene*	Xp11.21	X-linked	X-linked Dominant Protoporphyria (XLP)	<1
ALA Dehydratase (ALAD)	*ALAD Gene*	9q34	Autosomal Recessive	ALAD Deficiency Porphyria (ADP)	<0.0001
PBG Deaminase/ Hydroxymethylbilane Synthase (HMBS)	*HMBS Gene*	11q23.3	Autosomal Dominant	Acute Intermittent Porphyria (AIP)	5.4
Uroporphyrinogen III Cosynthase (UROS)	*UROS Gene*	10q26.2	Autosomal Recessive	Congenital Erythropoietic Porphyria (CEP)	<1
Uroporphyrinogen Decarboxylase (UROD)	*UROD Gene*	1p34.1	Autosomal Recessive	Hepatoerythropoietic Porphyria (HEP)	<1
Uroporphyrinogen Decarboxylase (UROD)	*UROD Gene*	1p34.1	Autosomal Dominant	Familial Porphyria Cutanea Tarda (f-PCT)	0.2 - 20
Coproporphyrinogen Oxidase (CPOX)	*CPOX Gene*	3q11.2	Autosomal Dominant	Hereditary Coproporphyria (HCP)	0.2
Protoporphyrinogen Oxidase (PPOX)	*PPOX Gene*	1q23.3	Autosomal Dominant	Variegate Porphyria (VP)	3.2
Ferrochelatase (FECH)	*FECH Gene*	18q21.31	Autosomal Recessive	Erythropoietic Protoporphyria (EPP)	9

*Prevalence data based on European countries.

Figure 1: Heme biosynthetic pathway with key enzymes, genes, inheritance patterns, and porphyria subtypes.

Figure *1* presents an overview of the heme biosynthesis pathway, highlighting the enzymatic steps, the corresponding causative genes, their chromosomal locations, mode of inheritance, and the associated porphyria subtypes (Ruthirago et al., 2016). Prevalence data are also shown to reflect the rarity and distribution of these disorders across various populations (Neeleman et al., 2020).

Accurate diagnosis of porphyria requires integration of clinical assessment with biochemical and molecular analyses to differentiate between the subtypes. While urine porphyrin profiles serve as an important preliminary screening tool, further confirmation via plasma porphyrins and genetic testing is often necessary, especially in ambiguous or overlapping presentations (Di Pierro et al., 2021).

Diagnostic approaches in Malaysia remains limited, with a scarcity of comprehensive biochemical and genetic data. The standard practice primarily relies on urinary porphyrin analysis, which, although useful for initial screening, lacks specificity in differentiating porphyria subtypes (Ogun et al., 2023). Advances in high-performance liquid chromatography (HPLC) and next-generation sequencing (NGS) technologies have enabled more precise characterisation through targeted molecular analysis. However, local application and validation of these tools remain underexplored, likely due to the rarity of cases, limited clinical awareness, resource constraints, and the absence of structured diagnostic guidelines and local genetic data.

This study aims to characterise porphyria subtypes among Malaysian patients by integrating clinical presentation with biochemical profiling of urinary and plasma porphyrins and genetic analysis. Specifically, we evaluate (i) the urinary and plasma porphyrin profiles using HPLC, and (ii) the underlying gene variants using targeted single-gene sequencing, whole-exome sequencing (WES), and multiplex ligation-dependent probe amplification (MLPA). The findings of this study aim to improve

the diagnostic pathway for porphyria and guide future implementation of diagnostic algorithms in Malaysian clinical practice.

METHODS

Study Design and Population

This was a cross-sectional descriptive study conducted from February 2022 to June 2024 at the Specialised Diagnostic Centre (SDC), Institute for Medical Research (IMR), Malaysia. Patients with clinical suspicion of porphyria—based on clinical symptoms and initial urine screening—were recruited. Cases with significant biochemical findings, such as elevated porphobilinogen or δ-aminolevulinic acid (D-ALA), were selected and recalled for plasma and molecular testing. Foreign nationals, forensic cases, and poor-quality samples were excluded.

Sample Collection and Processing

Random urine and blood specimens were collected and protected from light during collection and transport. Urine samples were analysed for total and fractionated porphyrins using high-performance liquid chromatography (HPLC) with a fluorescence detector. Heparinised plasma was analysed using a modified HPLC method for porphyrin quantification. 6 mL of whole blood in EDTA tubes was collected for genomic analysis.

Biochemical Analysis

Urinary porphyrin profiling included total porphyrins and fractionation into uroporphyrin, heptacarboxyporphyrin (hepta-), hexacarboxyporphyrin (hexa-), pentacarboxyporphyrin (penta-), coproporphyrin I (copro I), and coproporphyrin III (copro III) using Chromsystems Urine Porphyrin Kit. Results were expressed as nmol/L urine and interpreted against the laboratory reference intervals shown in Table 1. A ≥ two-fold increase above the upper reference limit for any relevant analyte was considered significantly elevated and indicative of a biochemical pattern consistent with porphyria subtypes, in accordance with current diagnostic guidelines (Di Pierro et al., 2021). Plasma porphyrin analysis followed a modified urinary method. This newly developed plasma protocol was validated in-house, and reference limits were established using healthy control samples.

Table 1: Reference intervals for urinary porphyrin fractions (nmol/L urine) used for interpretation of HPLC results.

Porphyrin Analyte	*Reference interval (nmol/L urine)**
Uroporphyrin	< 24
Heptacarboxyporphyrin	< 4
Hexacarboxyporphyrin	< 3
Pentacarboxyporphyrin	< 5
Coproporphyrin I	< 40
Coproporphyrin III	< 75
Total Porphyrins	<150

*According to the reference ranges of Chromsystems urine porphyrin kit verified in 2016

Genetic Analysis

Genomic DNA was extracted from EDTA blood, and DNA quality and quantity were determined. All samples underwent targeted *HMBS* gene sequencing. Cases that had negative *HMBS* mutations underwent WES, and analysis targeting eight primary porphyria genes (*ALAD, ALAS2, CPOX, FECH, HMBS, PPOX, UROD, UROS*) and three related genes (*CLPX, GATA1, HFE*). All pathogenic variants were confirmed by Sanger sequencing. Samples with no pathogenic variants

identified via WES were further analysed using multiplex ligation-dependent probe amplification (MLPA) to detect copy number variations (CNVs) using commercial kits (MRC Holland).

RESULTS

A total of 13 patients were included in the study, with a majority being female (n=9, 69.2%) and the remainder male (n=4, 30.8%). Most patients were under 40 years old, with the highest proportion in the 0–20 years age group (n=4, 30.8%), followed by those aged 21–30 and 31–40 years (n=3 each, 23.1%). By ethnicity, Malays accounted for the largest group (n = 5, 46.2%), followed by Chinese (n = 4, 30.8%), Indian (n = 1, 7.7%), and the remainder comprising Bumiputera Sabah (n = 2, 15.4%). Regionally, patients were predominantly from Borneo (n=4, 30.8%), followed by the Central Peninsular region (n=3, 23.1%), while other regions each contributed 15.4% of cases (Figure 2).

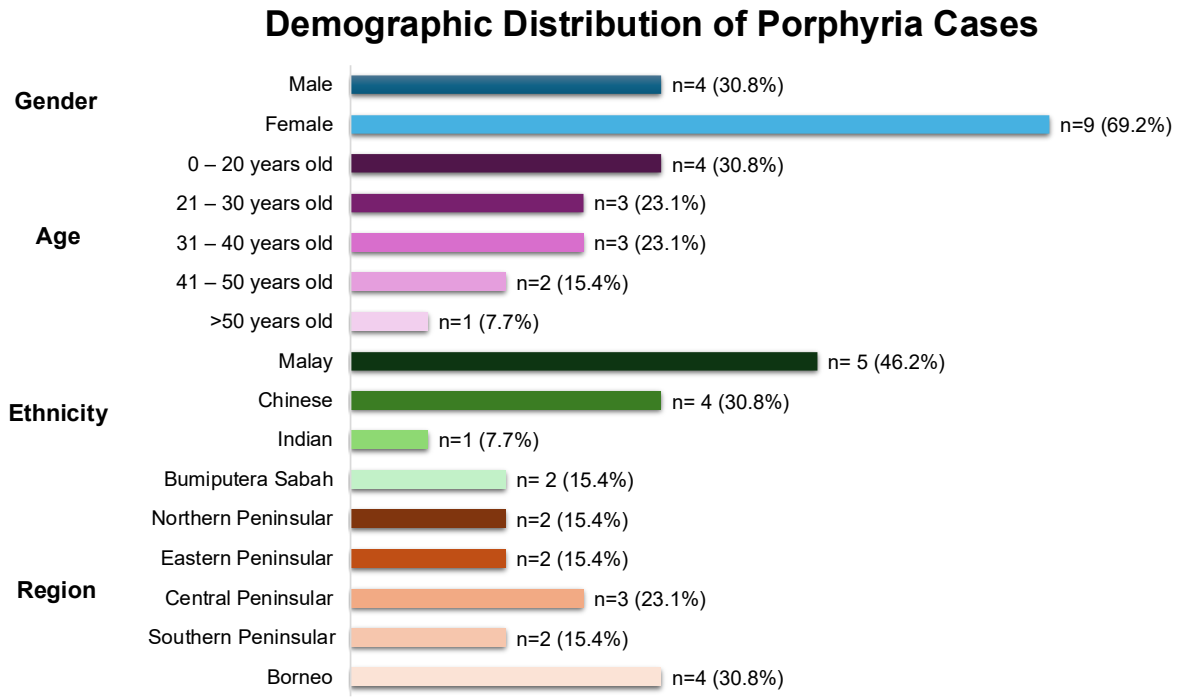


Figure 2: Demographic distribution of porphyria cases by gender, age group, ethnicity and region in Malaysia (N=13)

All patients (N=13) had elevated total urinary porphyrins. The levels varied between mild, moderate, and marked elevation, with higher levels correlating with the severity of clinical symptoms. Both urine and plasma porphyrin profile findings are shown in Figure 3. The most prominent urinary porphyrins were uroporphyrin (red) and coproporphyrin I and III (light and dark blue), indicating marked accumulation along the heme biosynthesis pathway.

A local reference interval for total plasma porphyrins was verified during this study using specimens from healthy volunteers (n = 61). The 95th percentile upper reference limit was established at < 16.6 nmol/L of total porphyrins, with individual porphyrin fractions defined as follows: uroporphyrin <7.9 nmol/L, heptacarboxyporphyrin <2.2 nmol/L, hexacarboxyporphyrin <3.0 nmol/L, pentacarboxyporphyrin <2.5 nmol/L, coproporphyrin I <3.5 nmol/L, and coproporphyrin III <4.3 nmol/L. All patient results were interpreted against these verified limits.

Out of thirteen cases, nine (n=9/13) were paired with plasma profiling. In the plasma profile, only a few patients (notably Cases 1, 2, 5 and 9) demonstrated detectable levels of porphyrins. Plasma

uroporphyrin was the dominant fraction in these cases, particularly Case 5, which mirrored the urinary profile, while the remaining five cases showed non-specific findings.

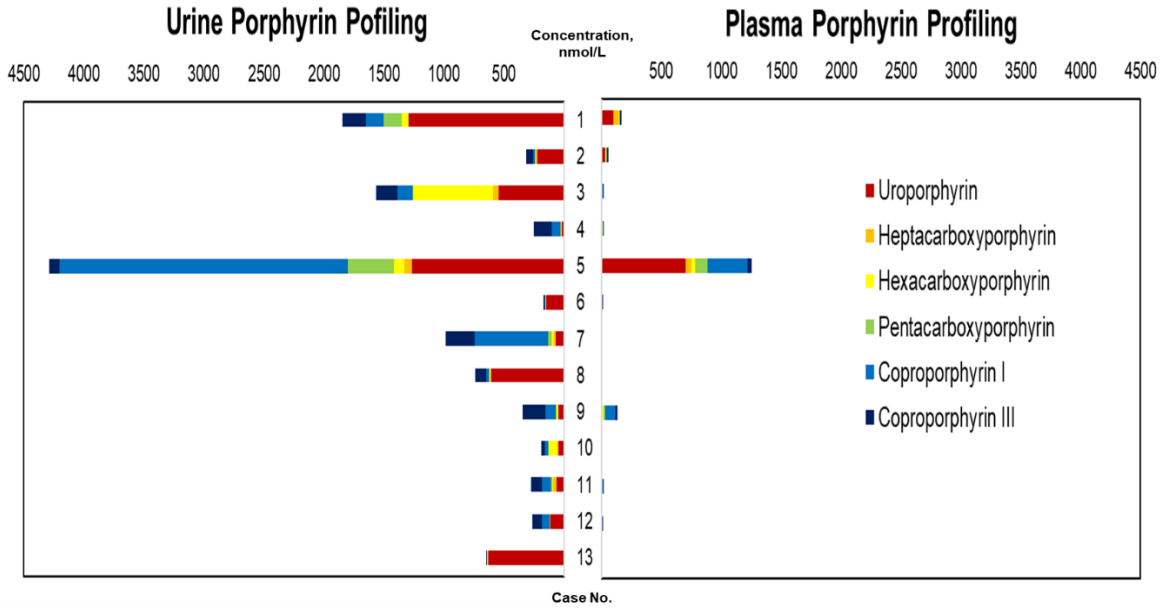


Figure 3: Combined Stacked bar graph showing porphyrin fractionation in urine and plasma of each case. No plasma porphyrin profiling for Case 7,8,10 & 13.

Molecular analysis revealed one case with *HMBS* gene variant, six cases with *FECH* gene variant, and one case of *HFE* gene variant, all detected by sequencing with no deletions/duplications (Table 2). No pathogenic variants were detected by WES in the remaining five cases (Case 9,10,11,12, and 13).

Table 2: Summary of Genetic Findings in Porphyria Cases

Case No.	Case Info	Gene Mutation	Genotyping	Zygosity	Pathogenicity	Inheritance
1	19 years old, Female	*HMBS gene*	c.673C>T, p.(Arg225*)	Heterozygous	Pathogenic	AD
2	42 years old, Female	*FECH gene*	c.315-48T>C, p.(?)	Homozygous	Likely Pathogenic	AR
3	23 years old, Female	*FECH gene*	c.315-48T>C, p.(?)	Homozygous	Likely Pathogenic	AR
4	20 years old, Female	*FECH gene*	c.315-48T>C, p.(?)	Heterozygous	Inconclusive	AR
5	52 years old, Male	*FECH gene* concurrent *G6PD gene*	c.315-48T>C, p.(?) c.1360C>T, p.(Arg454Cys)	Heterozygous Hemizygous	Inconclusive	AR XLR
6	28 years old, Male	*FECH gene*	c.315-48T>C, p.(?)	Heterozygous	Inconclusive	AR
7	15 years old, Female	*FECH gene*	c.315-48T>C, p.(?)	Heterozygous	Inconclusive	AR
8	28 years old, Male	*HFE gene*	c.187C>G, p.(His63Asp)	Heterozygous	Pathogenic	AR

Note: AD, autosomal dominant; AR, autosomal recessive; XLR, X-linked recessive

DISCUSSION

The demographic distribution in this study revealed a female predominance (69.2%), which is consistent with prior literature indicating a higher incidence of certain porphyria subtypes, such as AIP, in females (Katrin Baumann, 2022). This gender disparity may be influenced by hormonal factors, such as the role of progesterone in precipitating acute attacks, particularly during the reproductive years, as progesterone can up-regulate hepatic ALA-synthase-1 and increase heme precursor production, thereby triggering neurotoxic porphyrin accumulation (Bonkovsky et al., 2019; Vassiliou, 2023).

Age-wise, a notable proportion of patients (76.9%) were under 40 years old, with the highest frequency seen in the 0–20 age group. This early-onset pattern may reflect not only the genetic nature of certain subtypes but also greater clinical awareness of porphyria in recent years, supported by improved physician education, availability of HPLC-based porphyrin screening, and clearer referral pathways to specialised centres, all of which facilitate earlier recognition and testing (Di Pierro et al., 2021). For EPP in particular, symptoms like painful photosensitivity are often present in childhood or adolescence, prompting earlier diagnostic investigations (Minder et al., 2024).

Geographically, the higher number of cases from Malaysia Borneo (30.8%) and the Central Peninsular (23.1%) could reflect regional referral trends or population density; however, this finding may also suggest possible underdiagnosis in other regions due to limited awareness or access to clinician expertise. Further studies with larger populations are warranted to determine whether these trends reflect true regional variations in porphyria prevalence or healthcare accessibility.

Ethnicity-wise, the majority of patients were Malay, consistent with Malaysia's overall population structure and the ethnic composition of the referring hospitals. Although the small sample size limits firm conclusions, this distribution likely mirrors national demographics and healthcare-access patterns rather than a true ethnic predisposition. Nonetheless, potential underdiagnosis in minority groups cannot

be excluded, and larger population-based studies are needed to clarify any ethnic differences in porphyria prevalence.

For biochemical testing, the fractionation of porphyrin in urine and plasma serves as preliminary screening test for the diagnosis of hepatic porphyria, including Acute Intermittent Porphyria (AIP), Porphyria Cutanea Tarda (PCT), Hereditary Coproporphyria (HCP), or Variegate Protoporphyria (VP). It also helps to differentiate porphyria subtypes and guide the selection of second-line testing (Di Pierro et al., 2021). However, this study showed limited utility in detecting erythropoietic type of porphyria detection, as specific biomarkers, the protoporphyrin are not used in this study. Therefore, alternative methods and specimens such as bile or feces, where protoporphyrin are more concentrated, are required for biochemical diagnosis of Erythropoietic Protoporphyria (EPP) (Minder et al., 2024).

In this study, urine profiling demonstrated more distinctive porphyrin patterns compared to plasma. The lower concentrations of porphyrin analytes observed in plasma may be partly attributed to the hydrophobic nature of certain porphyrins in biological fluids (Keyfi et al., 2024), though this is not the sole explanation. The urine porphyrin assay in this study specifically detects the more water-soluble porphyrin species—uroporphyrin, hepta-, hexa-, penta-, and coproporphyrin I and III—which are efficiently excreted via the kidneys and often present at higher concentrations in urine samples from individuals with porphyria. In contrast, other porphyrin compounds may bind to the hydrophobic plasma proteins or lipids, reducing their free concentrations and affecting their detectability (Macours & Cotton, 2006). Additionally, factors such as porphyrin–heme binding, differences in excretion rates, and the diverse chemical properties of individual porphyrin species (e.g., varying hydrophobicity) contribute to their distribution in biological fluids and influence their quantification using HPLC (Colombi et al., 1983; Macours & Cotton, 2006).

Nevertheless, the markedly elevated levels of porphyrins in urine are strongly suggestive of true porphyria disorders. Patients with more severe clinical symptoms also exhibited higher urinary porphyrin concentrations, suggesting a correlation between urinary porphyrin levels and disease severity (Stölzel et al., 2019). Plasma porphyrin profiling proved useful in identifying AIP and PCT (Hindmarsh et al., 1999). The plasma analysis provides two main advantages: first, blood is easier and faster to collect than urine for emergency screening of an acute attack of porphyria. Second, as kidney disease is a common feature in acute hepatic porphyria (AHP), measurement in plasma samples allows for screening and/or follow-up in anuric or oligoanuric patients (Pallet et al., 2018; Poli et al., 2023).

Among the 13 patients, Case 1 was diagnosed with AIP based on concordant biochemical and molecular findings. The patient showed markedly elevated urinary PBG and D-ALA with urine and plasma porphyrin patterns consistent with the early heme biosynthesis blockade. Molecular analysis identified a pathogenic nonsense variant in *HMBS* c.673C>T, causing truncated enzyme and loss of activity, leading to neurotoxic precursor accumulation (Aarsand et al., 2024). The variant aligns with the molecular mechanism of autosomal dominant AIP, characterised by acute neurovisceral attacks often triggered by hormonal changes, certain drugs, or fasting (Linenberger & Fertrin, 2020).

Case 2 and 3 had EPP with homozygous *FECH* c.315-48T>C, a reduced-penetrance pathogenic variant that decreases ferrochelatase activity and causes variable expression. Both showed elevated porphyrins and neurovisceral symptoms, including abdominal pain and seizures, instead of typical photosensitivity, despite the variant's role in >90% of compound heterozygous EPP cases (Bonkovsky & Rudnick, 2025).

While most EPP cases are compound heterozygotes with one pathogenic variant and the c.315-48T>C, homozygotes may have milder or subclinical form of the disease, highlighting the need to integrate molecular, clinical, and biochemical data for diagnosis (Barman-Aksoözen et al., 2017). Diagnosis of EPP typically requires quantification of free protoporphyrin in erythrocytes or plasma, as this hydrophobic molecule is not excreted in urine. In this study, plasma porphyrin profiling showed only mild elevations in one of the EPP-confirmed cases, reflecting the limited sensitivity of plasma-based

screening. This further emphasises the need for erythrocyte porphyrin quantification or fecal porphyrin analysis to accurately confirm EPP (Minder et al., 2024).

Four patients (Case 4,5,6 and 7) were heterozygous for the *FECH* c.315-48T>C variant, a common hypomorphic allele (cause a partial reduction – nota a complete loss); that by itself does not typically cause EPP and is often found in asymptomatic individuals. These cases were therefore considered inconclusive based on molecular results alone.

Interestingly, Case 5 showed the highest total urinary porphyrin levels, with marked increases in multiple fractions (uroporphyrin, coproporphyrins I and III, and intermediate carboxylated porphyrins). This was unexpected given the presence of only a single heterozygous *FECH* c.315-48T>C variant, typically of low-penetrance and insufficient to cause overt EPP. The patient also had a hemizygous *G6PD* c.1360C>T variant, which, while not directly linked to porphyria, may have increased oxidative stress and hemolysis, increasing heme turnover and upregulating porphyrin precursor synthesis, thereby contributing to porphyrin accumulation in the setting of an underlying heme biosynthesis defect (Varghese et al., 2021).

Case 8 carried a heterozygous *HFE* c.187C>G variant, linked to hereditary hemochromatosis (HH) and often seen in PCT (Bygum et al., 2003; Singal, 2022). This mutation can promote iron overload, a known trigger for PCT by inhibiting hepatic uroporphyrinogen decarboxylase (UROD) activity, leading to urinary accumulation of highly carboxylated porphyrins and chronic cutaneous symptoms (Elder, 2012; Singal, 2022). Environmental and hormonal factors (e.g., alcohol, estrogen, smoking, hepatitis C, liver disease) can further increase PCT penetrance (Adams et al., 2020). While heterozygous *HFE* alone is insufficient to cause PCT, it may predispose to disease in the presence of additional triggers (Leaf & Dickey, 2024). However, no specific haemochromatosis work-up (e.g., serum iron studies or ferritin) was performed during this study, so iron overload could not be confirmed.

No pathogenic variants were found in the porphyria-related genes screened for the remaining five patients. These negative results indicate that their symptoms might stem from other genetic or metabolic conditions, or acquired forms of porphyria. In such cases, environmental or lifestyle factors like hepatotoxic medications, infections, alcohol use, or toxin exposure should be considered in the differential diagnosis (Elder, 2012) .

EPP seemed more frequent in our study population, especially when identified through WES, aligning with European data (Linenberger & Fertrin, 2020; Neeleman et al., 2020). However, our biochemical analysis demonstrated limited ability to detect protoporphyrin, the primary biomarker of EPP. Most EPP-related cases (e.g., 3, 4, 6, 11, and 12) showed very low or undetectable plasma porphyrins, underscoring the limitations of plasma profiling for erythropoietic porphyrias (Minder et al., 2024). The inconclusive or negative genetic results in 9 of 13 patients further highlight the constraints of current genetic testing, particularly in detecting deep intronic, regulatory, or structural variants as well as potential pathogenic changes in genes not covered by the targeted analysis. Although WES was included in this study, variants outside the initial analysis scope may remain undetected, underscoring the need for reanalysis guided by comprehensive clinical data to improve variant identification. These findings reinforce the critical need for integrating molecular data with biochemical profiles and thorough clinical evaluation for accurate diagnosis and effective patient management.

The higher detection of EPP and EPP carriers over AIP reflects a significant diagnostic gap, particularly the inability of current assays to capture protoporphyrin. Future work should prioritise the development and implementation of methods for detecting protoporphyrin in erythrocytes, plasma, or faeces, with spectrophotometric scanning of plasma or stool offering a potentially simpler and cost-effective screening approach. Finally, the identification of *FECH*, *HMBS*, and *HFE* variants add valuable insight into the genetic landscape of porphyria in Malaysia, contributing to an important but still underexplored area of research.

CONCLUSION

This study highlights the value of integrating biochemical and molecular tools in the diagnosis of porphyria in Malaysian patients. The presence of low-penetrance or absent variants in some patients underscores the importance of thorough clinical evaluation and consideration of environmental triggers when interpreting findings. While urine and plasma porphyrin testing remain the primary initial tools, subtype-specific biochemical differences emphasise the need for more sensitive and specific methods, particularly for detecting protoporphyrin in erythropoietic porphyrias. The integration of next-generation sequencing holds promises for advancing precision medicine in porphyria, allowing for comprehensive variant detection, refined genotype–phenotype correlations, and personalised management. Ultimately, the establishment of a clear diagnostic algorithm or clinical guideline tailored to our local healthcare settings is needed. This would optimise test selection based on clinical presentation, enhance diagnostic accuracy, improve resource utilisation, and lead to better patient outcomes in porphyria care. Despite its limitations, this study serves as a pioneer effort and provides a foundation for future large-scale population-based studies in Malaysia.

ACKNOWLEDGEMENTS

We thank our Director-General of Health Malaysia and the Institute for Medical Research Director for permission to publish this paper. We also thank the Biochemistry and Molecular Unit's staff for their direct or indirect contribution to the laboratory work.

FUNDING

This research received funding from the Ministry of Health (MOH) Research Grant (MRG)

AUTHORS' CONTRIBUTION

Sofwatul Mukhtaroh, N. and Anasufiza, H. conceptualised and designed the study. Muhammad Rezwan, R. conducted the experiments and compiled the data. Sofwatul Mukhtaroh, N. and Ahmad Kamal Maula, A.R. were responsible for the methodology, participant recruitment, sample collection, and validation. Lua, S.H. and Yusnita, Y. carried out the genetic analysis, validation, and interpretation. Azzah Hana A.Y., Yusnita, Y., and Anasufiza, H. contributed significantly to result interpretation. Sofwatul Mukhtaroh, N. spearheaded the manuscript writing. All authors critically reviewed the manuscript, offered substantial input into data interpretation, and made significant contributions to shaping the analysis and refining the final draft.

CONFLICT OF INTEREST DECLARATION

We affirm that there is no Conflict of Interest among the author(s) concerning the subject matter or materials discussed in this manuscript. We further certify that the article represents the original work of the Authors and Co-Authors. The manuscript has not been previously published and is not currently under consideration for publication elsewhere. This research/manuscript has neither been submitted for publication nor published in whole or in part elsewhere. We attest that all authors have made significant contributions to the work, ensuring the validity and legitimacy of the data and its interpretation, thereby warranting its submission to the Malaysian Journal of Clinical Biochemistry.

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